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Electrocardiographic Responses of Rat Fetuses with Clamped or Intact Umbilical Cords to Acute Maternal Uterine Ischemia

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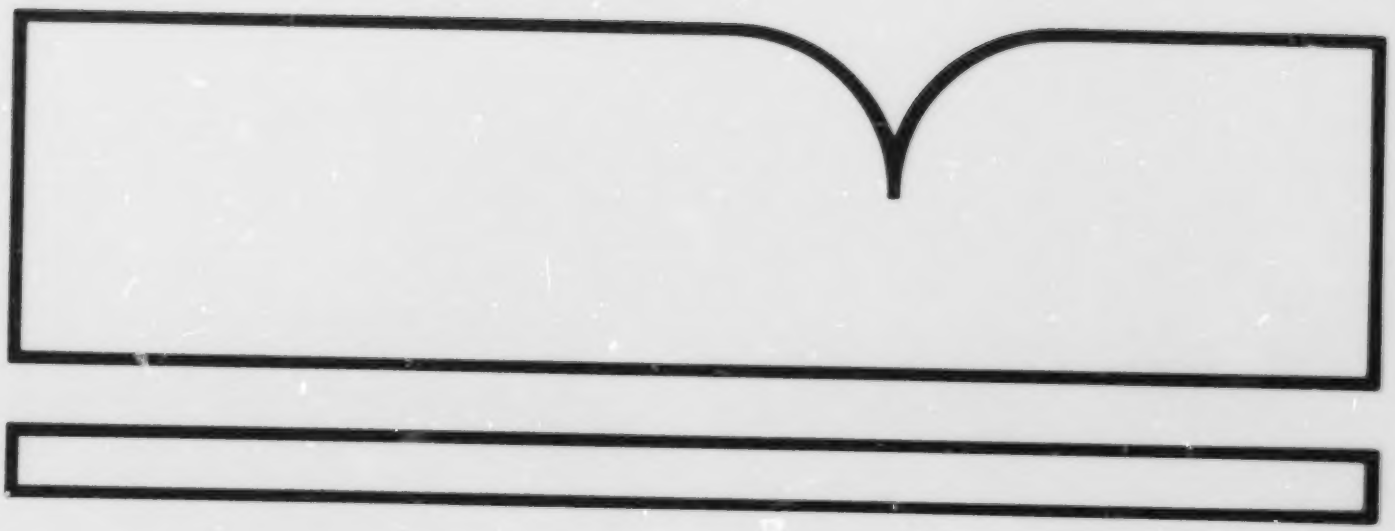

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16. ABSTRACT

Uterine ischemia results in severe cardiac disturbances in the fetus. It has been postulated that these effects are due to interaction with the ischemic uterus or placenta and not due to hypoxia or build up of metabolites in the fetus. The fetal cardiac responses to uterine clamping and umbilical cord clamping were compared in the rat using electrocardiography (ECG). On day 21 of gestation fetuses from the same pregnant rat were exposed to umbilical clamping, uterine clamping or no clamping in a total of 14 pregnant rats. ECGs were recorded for each fetus immediately after clamping and at 10, 20 and 30 minutes after clamping. Immediately after clamping, the uterine clamping group showed severe sinus bradycardia. At all other observation times fetuses exposed to uterine or umbilical clamping showed bradycardia and other ECG changes typically associated with hypoxia. Therefore, umbilical clamping protected the fetuses from the adverse effects of uterine clamping for only a very short time.

17. KEY WORDS AND DOCUMENT ANALYSIS

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Electrocardiographic responses of rat fetuses with clamped or intact umbilical cords to acute maternal uterine ischemia

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Research Triangle Park, North Carolina

Uterine ischemia results in severe cardiac disturbances in the fetus. It has been postulated that these effects are due to interaction between the fetus and the ischemic uterus or placenta, and not to hypoxia or buildup of metabolites in the fetus. Rat fetal cardiac responses to uterine clamping and umbilical cord clamping were compared by electrocardiography. On day 21 of gestation, fetuses in a total of 14 pregnant rats were exposed to umbilical clamping, uterine clamping, or no clamping. Electrocardiograms were recorded for each fetus immediately after clamping and at 10, 20, and 30 minutes after clamping. Immediately after clamping, the uterine clamping group alone showed severe sinus bradycardia. At all other observation times, fetuses exposed to uterine or umbilical clamping showed bradycardia and other electrocardiographic changes typically associated with hypoxia. Therefore, umbilical clamping protected the fetuses from the adverse effects of uterine clamping for only a very short time. (AM. J. OBSTET. GYNECOL. 147:795, 1983.)

In a pregnant animal, caffeine,[1] nicotine,[2] various drugs,[3] or pathologic conditions[4] may reduce normal blood flow to the uterus and, thus, to the embryo or fetus. A reduction in uterine blood flow induces immediate physiologic and biochemical disturbances in the embryo or fetus[4, 5] and may result in permanent damage to the offspring.[5, 6] Since embryonic or fetal hypoxia (oxygen deficiency) is perhaps the most obvious deleterious effect of a reduction in uterine blood flow, the assumption is often made that hypoxia is the causative factor both in the cardiac disturbances that follow such an insult and in any permanent damage that may result. However, simple hypoxia probably rarely occurs alone, and, depending upon the experimental design and animal used, embryonic or fetal hypoxia may be accompanied by nutritional restriction, hypercapnia, hypotension, acid-base disturbances, lactic acid accumulation, hyperkalemia, fluid disturbances, and bradycardia.[4]

Uterine clamping[7, 8] is a mechanical technique that has been used to study the effects of reduced uterine blood flow in experimental animals. This technique has been shown to induce fetal cardiac disturbances.[9] Millicovsky and DeSesso[10] demonstrated that clamping the umbilical cord prior to uterine clamping protected rabbit embryos from the cardiac disturbances that are induced by uterine clamping. Their results demonstrated that embryonic hypoxia alone was not sufficient to induce cardiac disturbances. They suggested that other factors, such as the release of some substance from the ischemic uterus or placenta, may be a more serious threat to the embryo than hypoxia. The present study was designed to expand upon the observations of Millicovsky and DeSesso by using another species at a later gestational age and by extending the observation time after clamping.

Methods

Ninety-day-old pregnant rats were obtained on days 12 to 14 of gestation from the Charles River Breeding Laboratory, Kingston, New York. The animals were housed individually in plastic cages with pine shavings bedding in a bioclean room with a temperature of 22° to 26° C and a 12-hour-light, 12-hour-dark cycle. Tap water and Purina rat chow were provided ad libitum.

Electrocardiograms were recorded with a Hewlett-Packard 7758B recording system equipped with an 8811A bioelectric amplifier and a 7758A paper chart recorder. The electrocardiographic signals were transmitted directly from this recording system to a four-channel FM cassette recorder. Electrodes were connected to the bioelectric amplifier by a Hewlett-Packard 1414B patient cable. Needle electrodes (Grass Instrument Company, Quincy, Massachusetts) were used for pregnant rats, and silver/silver chloride surface electrodes (In Vivo Metric Systems, Heraldsburg, California) were used for fetuses. Both for fetuses and adult rats, the electrodes were positioned for recording standard electrocardiographic leads I, II, and III, although lead II was usually used for analysis. A recording was taken from each lead at each

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Fig. 1. Method of uterine and umbilical clamping in the rat on day 21 of gestation. Hemostatic clamps were placed across one uterine horn. Although not shown in this drawing, the hemostatic clamps also occluded all vasculature to and from the clamped horn. Small bulldog clamps were placed across the umbilical cord of one fetus from each uterine horn. The four fetal treatments shown are: *Upper left*, umbilical and uterine clamping; *lower left*, uterine clamping; *upper right*, umbilical clamping, and *lower right*, control.

recording time. After completion of the experiment, electrocardiograms recorded on the cassette tapes were analyzed with a microcomputer system described previously.[11] Heart rate, PR interval, PT interval, QT interval, ST interval, P-wave duration, QRS complex duration, T-wave duration, ST segment length, and PR segment length were measured.

On day 21 of gestation, the pregnant rats were lightly anesthetized with Metofane (Pitman-Moore, Inc.), and then injected with Nembutal (36 mg/kg, intraperitoneally, Abbott Laboratories) to maintain anesthesia throughout the experimental period. An electrocardiogram was then recorded on the pregnant animal. A midline incision was made and the gravid uterus was exposed. Only animals with at least three live fetuses in each horn were used. An incision was made through each uterine horn at the ovarian end, and two fetuses per horn were removed and placed in paraffin retaining dishes. All membranes were carefully removed. Care was taken to ensure that the placental connection remained intact, and that there was as little strain as possible on the umbilical cord. Electrodes were then positioned and an electrocardiogram was recorded for each fetus. Next, hemostatic clamps were placed across the right uterine horn and its vasculature at the ovarian and cervical ends of the horn. The umbilical cord from one fetus in each uterine horn was then clamped with bulldog clamps, as shown in Fig. 1. Four fetal treatment groups were thus created: (1) control (no clamping), (2) umbilical and uterine clamping, (3) umbilical clamping, and (4) uterine clamping. The second group, umbilical and uterine clamping, was included to balance the experimental design; however, it could actually be considered as another umbilical clamping group because the umbilical clamp prevented any interaction between the fetus and the clamped uterine horn. The time of clamping was noted, and an electrocardiogram was immediately recorded for each fetus. Electrocardiograms were again recorded for the four fetuses at 10, 20, and 30 minutes after clamping. Another electrocardiogram was recorded for the pregnant animal at the end of the experimental period. This procedure was repeated for a total of 14 pregnant rats.

Data were analyzed with the General Linear Models Procedure of the Statistical Analysis System with the use of repeated measures analysis of variance. Least-squares means procedures were used to test for differences between treatment groups. The experimental design was double nested or split split plot. Treatments were nested within litters, and electrocardiographic recording times were nested within each fetus. Pregnant rats were used as blocking factors.

Results

In the pregnant animals and control fetuses, electrocardiographic wave intervals and heart rates did not change significantly over the 30-minute experimental period. In addition, no significant differences were found among the designated fetal treatment groups prior to clamping. Within 10 minutes after the clamps had been applied, longer PR intervals were observed in all clamping groups. In these groups, PR intervals continued to increase over the experimental period, and no differences were observed between uterine and umbilical clamping (Fig. 2). At the 20- and 30-minute recording times, all clamping groups had significantly longer QRS complex durations. The QRS complex duration of the umbilical clamping group was significantly

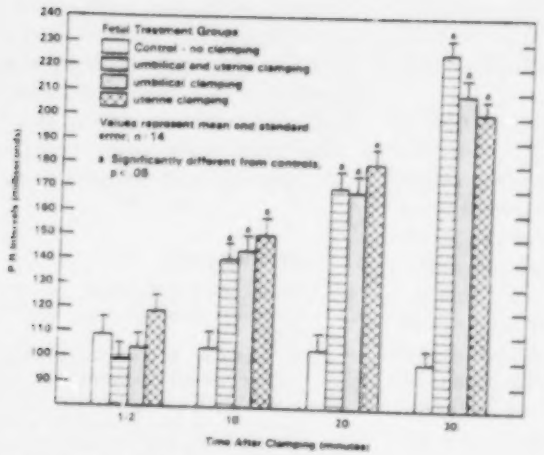

Fetal Treatment Groups
Control - no clamping
umbilical and uterine clamping
umbilical clamping
uterine clamping
Values represent mean and standard error, n = 14
a Significantly different from controls, p < .05
Time After Clamping (minutes)

Fig. 2. Effect of uterine and umbilical clamping on PR intervals in the rat fetus on day 21 of gestation. PR intervals became longer for all three clamping groups over the course of the experiment. Hypoxia is known to result in longer PR intervals.

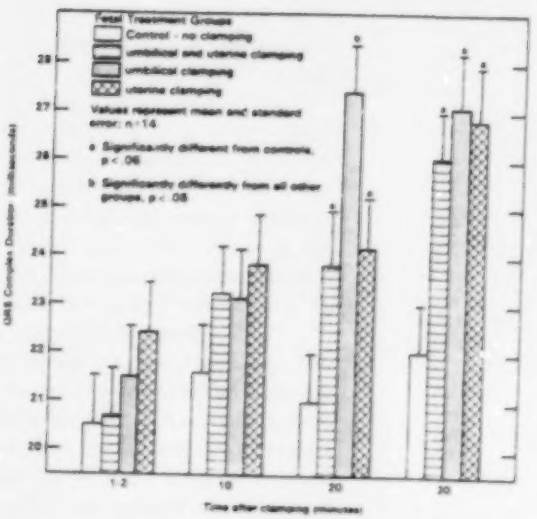
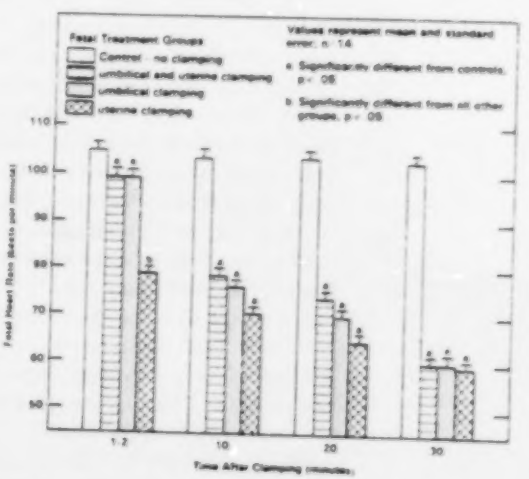

Fetal Treatment Groups
Control - no clamping
umbilical and uterine clamping
umbilical clamping
uterine clamping
Values represent mean and standard error, n = 14
a Significantly different from controls, p < .05
b Significantly differently from all other groups, p < .05
Time after clamping (minutes)

Fig. 3. Effect of uterine and umbilical clamping on QRS complex duration in the rat fetus on day 21 of gestation. Beginning at 20 minutes after clamping, the duration of the QRS complex was significantly longer in the three clamping groups than in the control group. The reason for the very long QRS complex duration in the umbilical clamping group at 20 minutes after clamping is unknown. Thirty minutes after the clamping procedure, there were no significant differences in the three clamping groups.

longer than that of any other group at the 20-minute recording period (Fig. 3). Immediately after clamping, the fetal groups with umbilical clamps had lower heart rates than did the control animals. In contrast, when the fetuses were examined immediately after clamping, the uterine clamping group had much more severe bradycardia than the umbilical clamping groups, although no other electrocardiographic irregularities were found at this time. Heart rates of all clamping groups continued to decline throughout the examination period, although differences between uterine and umbilical clamping groups were found only when the fetuses were examined immediately after clamping (Fig. 4).

Comment

Fetuses exposed to uterine or umbilical clamping are deprived of their normal blood exchange with the pregnant animal. The primary difference between these two clamping procedures is that the fetuses in clamped uterine horns with intact umbilical circulations are potentially exposed to substances released by the ischemic uterus or placenta. In the study by Millicovsky and DeSesso,[10] umbilical clamping protected rabbit embryos from the cardiac derangements induced by uterine clamping for a 10-minute observation period. Those authors hypothesized that the cardiac disturbances that they observed in the embryos exposed to uterine clamping were due to substances released by the ischemic uterus or placenta and not to embryonic hypoxia or a build-up of toxic metabolites in the embryo. For example, it has been hypothesized that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umbilical clamping
uterine clamping
Values represent mean and standard error, n = 14
a Significantly different from controls, p < .05
b Significantly different from all other groups, p < .05
Time After Clamping (minutes)

Fig. 4. Fetal rat heart rate response to uterine and umbilical clamping on day 21 of gestation. In the three clamping groups, fetal heart rates dropped over the course of the experiment, whereas control heart rates did not change significantly. When heart rates were recorded immediately after clamping (1 or 2 minutes), the uterine clamping groups were experiencing much more severe bradycardia than the two groups with umbilical clamps. This was the only evidence in this study that indicated that the umbilical clamps may have provided some protection from the effects of uterine clamping.

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uteroplacental ischemia may trigger the release of vasoactive compounds which are known to be both embryolethal and teratogenic[12–14] and to induce fetal cardiac disturbance.[9]

In the present study, umbilical clamping seemed to protect the fetuses from the adverse effects of uterine clamping only immediately after the clamping procedure. Ten minutes after clamping, all fetuses exposed to either uterine or umbilical clamping were experiencing bradycardia and other electrocardiographic changes commonly associated with hypoxia.[15] Millicovsky and DeSesso[10] observed rabbit embryos on day 12 of a 32-day gestational period, whereas the present study used rat fetuses on day 21 of a 22-day gestational period. The different gestational ages used in the two studies may provide the best explanation for the differences in experimental results. It is accepted that young embryos are less susceptible to hypoxic damage than are older fetuses.[4] An embryo or fetus exposed to reduced uterine blood flow may initially react to some substance released by the ischemic uteroplacental unit. Later, as the embryonic or fetal tissues become more hypoxic, typical changes associated with hypoxia may result. Because near-term fetuses are more susceptible to hypoxic damage than embryos, the fetuses may exhibit the hypoxic damage much more rapidly than the embryos.

Often electrocardiographic wave intervals vary inversely with heart rate.[16] In the uterine and umbilical clamping groups in this study, as heart rate became lower over the course of the experiment, the PR interval and QRS complex duration measurements became longer. In contrast, the immediate severe bradycardia that was found in the group exposed to uterine clamping alone was not accompanied by any other electrocardiographic irregularities. Sinus bradycardia, which is a slow heart rate with regular rhythm, often reflects a depression of the sinus pacemaker and commonly represents increased vagal tone.[17] This suggests that the very low rate found in the uterine clamping group immediately after clamping may represent an effect on the central nervous system rather than a direct effect on the heart. This provides evidence that the immediate severe bradycardia found in the uterine clamping group was induced by a different mechanism of action than the longer-term progressive bradycardia found in all clamping groups.

In conclusion, umbilical clamping seemed to protect the near-term rat fetus from the immediate severe bradycardia that was observed in fetuses exposed to uterine clamping. The umbilical clamping did not provide any long-term protection for the fetuses. At 10, 20, and 30 minutes after clamping, fetuses exposed to both uterine and umbilical clamping showed electrocardiographic changes typically associated with hypoxia. Evidence was found suggesting that the immediate severe bradycardia observed in the uterine clamping group was induced by a different mechanism of action than the longer-term progresssive bradycardia observed in all clamping groups.

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